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Methane, Carbon Dioxide, and Hydrogen Sulfide Production from the Terminal Methiol Group of Methionine by Anaerobic Lake Sediments

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A significant portion of the sulfide in lake sediments may be derived from sulfur-containing amino acids. Methionine degradation in Lake Mendota (Wisconsin) sediments was studied with gas chromatographic and radiotracer techniques. Temperature optimum and inhibitor studies showed that this process was biological. Methane thiol and dimethyl sulfide were produced in sediments when 1-μmol/ml unlabeled methionine was added. When chloroform (an inhibitor of one-carbon metabolism) was added to the sediments, methane thiol, carbon disulfide, and *n*-propane thiol were produced, even when no methionine was added. When ^{35}S-labeled methionine was added to the sediments in tracer quantities (1.75 nmol/ml), labeled hydrogen sulfide was produced, and a roughly equal amount of label was incorporated into insoluble material. Methane and carbon dioxide were produced from [*methyl*-^{14}C]methionine. Evidence is given favoring methane thiol as an intermediate in the formation of methane, carbon dioxide, and hydrogen sulfide from the terminal methiol group of methionine. Methionine may be an important source of sulfide in lake sediments.

In freshwater, where sulfate concentrations are much lower than in seawater, a significant portion of the sulfide in sediments can be derived from organic sulfur compounds, rather than by sulfate reduction. Nriagu (13), on the basis of carbon-sulfur ratios of the organic matter reaching Lake Mendota (Wisconsin) sediments, calculated that organic sulfur was the source of about half the sulfide in the sediments. During the summer, Lake Mendota has a dense cyanobacterial (blue-green algal) bloom, and members of this population, usually dead or senescent cells, settle to the bottom. In other studies in this laboratory (R. D. Fallon and T. D. Brock, unpublished data), it has been found that 0.2 to 0.8 g of protein/m^2 per day reaches the sediment during the summer, and as much as 1.5 g/m^2 per day settles in the autumn during algal decline. It is reasonable to expect that methionine and cysteine will be present in this protein and that these amino acids will be degraded in the sediments.

The degradation of cysteine to hydrogen sulfide is well known (9), but the anaerobic production of hydrogen sulfide from methionine is much less well characterized. The formation of homocysteine from methionine and hydrogen sulfide from homocysteine are well-known biochemical reactions, and Postgate (14) has suggested that this is the mechanism of hydrogen sulfide production from methionine. However, studies of microbial dissimilatory metabolism of methionine have found methane thiol, dimethyl disulfide, and dimethyl sulfide (9, 16) as products, rather than hydrogen sulfide.

There have been few studies of methionine breakdown in anaerobic habitats. Francis et al. (8) added methionine to soils incubated anaerobically and found methane thiol, dimethyl sulfide, dimethyl disulfide, and methyl thioacetate as products. When glucose was not added along with the methionine, no volatile products were detected. Salsbury and Merricks (15) found that methane thiol and dimethyl sulfide were produced from methionine added to rumen fluid. Carbon tetrachloride, a well-known inhibitor of one-carbon metabolism, did not inhibit methane thiol production from methionine, but did inhibit dimethyl sulfide production from methionine; it also inhibited methanogenesis. Carbon tetrachloride did not inhibit dimethyl sulfide formation from dimethyl acetothetin chloride, a sulfonium compound, and the authors concluded that dimethyl sulfide formation from methionine involved transfer of a methyl group, while dimethyl sulfide formation from sulfonium compounds did not.

Both of these studies, and our own initial studies on methionine decomposition in Lake

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Mendota sediments, involved the addition of methionine to a final concentration of at least 1 μmol/ml. It is unlikely that experiments involving the additions of such large amounts of methionine accurately simulate the response of the habitat to the much lower natural concentrations of this amino acid. Furthermore, in our earlier lake sediment studies, the production of hydrogen sulfide, a potentially important product, could not be monitored because the background sulfide concentrations in the sediments were already so high. Because of these limitations, we undertook studies of methionine decomposition involving [^{35}S]methionine and [*methyl*-^{14}C]methionine at a concentration (1.75 nmol/ml) three orders of magnitude lower than that used in the previous studies. The results obtained with this method were considerably different, as presented in this report.

MATERIALS AND METHODS

Sampling location and procedures. Lake Mendota (surface area, 3.9×10^7 m^2; volume, 4.9×10^8 m^3) is a hard water dimectic eutrophic lake with a marl bottom. From July until turnover in October, the water overlying the sediments contains hydrogen sulfide. Most of the samples studied were taken during this period, although there were similar results with samples obtained at other times of the year. The sediment dry weight is 15% of the wet weight, and about half of the dry weight is organic matter (22). The sediment contains large amounts of precipitated calcium and magnesium carbonates, and the pH is close to 7.0 (22). The acid volatile sulfide concentration is 800 μg/ml (5 mg/g [dry weight]), mostly as amorphous iron sulfide (13). Free sulfide in the pore waters is usually less than 1 μg of S per ml (13, 21). The sulfate concentration in the water column is 8 to 10 μg of S per ml; in the sediments it is at the limit of detection (<1 μg of 5 per ml) (21). These sediments actively produce methane during the entire year, although in winter months methane production is limited by low temperatures (22).

The sampling location was termed the "central station." This was the deepest section of the lake, with a water column depth of 23 m. Samples were taken with an Eckman dredge (Wildlife Supply Co., Saginaw, Mich.) and were immediately dispensed undiluted into neoprene-stoppered anaerobic tubes and stored under a nitrogen atmosphere at 4°C until use. The ferrous sulfide (7) and other redox buffers present in the sediments prevented harm to anaerobes by the brief exposure to oxygen while the sediments were dispensed (21). It was found that the sediment samples could be stored at least 2 weeks without any loss of activity.

Gas chromatographic analysis of volatile sulfur compounds. Volatile sulfur compounds were identified and quantified with a gas chromatograph (model 419; Packard Instrument Co., Downers Grove, Ill.), fitted with a flame photometric detector, which is specific for and sensitive to nanogram quantities of sulfur compounds. The Teflon column of Stevens et al. (18) was used, and the operating conditions were those described by Banwart and Bremner (1). Calibration standards of the compounds were prepared in one of two ways. If the substance was gaseous, a known volume of the pure gas was diluted in serum vials capped with Teflon Mininert valves (Precision Sampling Corp., Baton Rouge, La.). If the substance was a liquid, such as dimethyl sulfide, several milliliters of the liquid were placed in a serum vial, which was then sealed with a rubber stopper. The vial was evacuated, and a known volume of the headspace was removed with a gas-tight syringe and diluted in serum vials capped with Mininert valves. The concentration was then determined from the vapor pressure of the substance at the ambient temperature (20). Propane thiol concentration was estimated from the calibration curve for methane thiol, and, although it has been found that a separate calibration curve must be determined for every sulfur compound (1), a rough approximation was obtained this way.

The retention times (in seconds) for the various compounds were: hydrogen sulfide, 88; methane thiol, 12; dimethyl sulfide, 180; carbon disulfide, 215; propane thiol (tentative), 280; and dimethyl disulfide, 795.

Metabolism of unlabeled sulfur compounds. To study the metabolism of unlabeled sulfur compounds, 2-ml samples of sediment were dispensed by syringe into 10-ml serum vials, and 0.1 ml of either 20-μmol/ml L-methionine or 20-μmol/ml L-cysteine HCl (neutralized to pH 7) was added so that the final concentration was 1 μmol/ml. To controls, 0.1 ml of distilled water was added. The vials were gassed with prepurified nitrogen gas (passed over heated copper filings) and sealed with Teflon Mininert valves. To some samples, 1 μl of chloroform was added by microsyringe through the Mininert valve, so that the final concentration was 750 μg/ml. The samples were incubated at 20°C for 4 days, and a 1-ml sample of the headspace was taken with a gas-tight syringe and analyzed for volatile sulfur compounds by gas chromatography.

Metabolism of [^{35}S]methionine by lake sediments. To examine the metabolism of [^{35}S]methionine by lake sediments, 1 ml of sediment each was added by syringe to 10-ml serum vials preflushed with N_2, and then 50 μl of either distilled water or distilled water containing an addition was added. The mixture was gently blended with a Vortex mixer, and the sediment, when an addition was present, was preincubated for 2 h at 0°C under a nitrogen atmosphere to ensure adequate contact between the sediments and the additions. After this period, 25 μl containing 1.75 nmol of [^{35}S]methionine (aqueous solution, initial activity 580 mCi/mmol; Amersham/Searle, Arlington Heights, Ill.) was added. The vials were then gassed with nitrogen while being sealed with Tuf-Bond Teflon-coated silicone septa (Pierce Chemical Co., Rockford, Ill.) with crimp-on aluminum seals. The removal of the gassing needle and the crimping on of the stopper was done with one motion; with practice, this procedure (and the large redox buffer capacity of the sediments) kept the sediments sufficiently anaerobic that methane was produced, and there was no visible oxidation of the sediments even after 4 days of incubation. The Tuf-Bond disks were impermeable to oxygen as long as they were not pierced by a syringe needle, and the Teflon coating ensured that no volatile

organic sulfur compounds would be absorbed into the septum, a problem with rubber septa.

After addition of the label, the sediments were usually incubated for 2 to 4 h at 15°C (the highest in situ temperature reached by the lake sediments). The different treatments were done in duplicate, and each experiment reported was repeated at least once with similar results.

To determine the fate of the label after incubation, the following fractionation procedure was devised. After the incubation period, 0.1 ml of 1% $FeSO_4 \cdot 5H_2O$ (neutralized to pH 7.0 and stored under a hydrogen atmosphere) was added to the vials by syringe through the septum. Preliminary experiments have shown that the ferrous iron will react with free hydrogen sulfide, forming amorphous ferrous sulfide, but will not trap methane thiol or other organic sulfur compounds. Nitrogen gas was then bubbled through the sediment and through Teflon tubing into a solution of 3% $HgCl_2$, which traps volatile organic sulfur compounds (16). The nitrogen flow was about 1 ml/s, and the samples were bubbled for 5 min. A sample of the $HgCl_2$ solution was counted in Aquasol scintillation cocktail (New England Nuclear Corp., Boston, Mass.). All ^{35}S radioactivity was counted with a Tri-Carb 3375 scintillation spectrometer (Packard) with the window set at 40 to 1,000 and the gain at 12%. Quench correction was by the channels ratio method. The counts in the $HgCl_2$ solution were considered to be the volatile organic sulfur compounds that could be freed from the sediment.

After bubbling through the $HgCl_2$ solution, the sample was acidified by the addition of 1.0 ml of either 3.6 N H_2SO_4 or 3.0 N HCl, and the hydrogen sulfide liberated was trapped by bubbling through an acidified zinc acetate solution (6) for 10 min. This removed over 98% of the acid-labile sulfide present in the sediments. The acidification of the sediments also served to stop all biological reactions. A sample of the zinc sulfide suspension was counted in Aquasol and was considered to be the label converted to hydrogen sulfide. This was verified by acidifying a sediment sample after incubation with [^{35}S]methionine and injecting a sample of the headspace through the gas chromatograph column. At various time intervals, the column effluent was trapped in mercuric chloride, which traps both hydrogen sulfide and volatile organic sulfur compounds, and the radioactivity in the fractions from the column was counted. With this method, it was found that the radioactivity coincided with the hydrogen sulfide peak.

The remaining label was fractionated in the following manner. The vial was opened and the contents were washed into a centrifuge tube with 4 ml of distilled water. The sediment suspension was centrifuged at 8,000 × *g* for 10 min. A sample of the supernatant was counted in Aquasol, and this was the soluble radioactivity. The pellet was resuspended in distilled water to a total volume of 5 ml and blended with a Vortex mixer until the suspension was even. A 0.1-ml sample of this suspension was filtered through a 0.45-μm-pore membrane filter (Gelman Instrument Co., Ann Arbor, Mich.), followed by two washes of distilled water. The filters were dried and counted in a toluene-based scintillation cocktail. The counts in the pellet were the insoluble counts or those bound to the sediment.

In summary, after incubation, the label was divided into four fractions: free volatile organic sulfur compounds, hydrogen sulfide, the soluble fraction, and the insoluble fraction.

Metabolism of [*methyl*-^{14}C]methionine. The procedure for studying the metabolism of [*methyl*-^{14}C]methionine was similar to that for [^{35}S]methionine, except that 1.75 μmol of [*methyl*-^{14}C]methionine (53 mCi/mmol, Amersham/Searle) was added to 1 ml of sediments. After incubation with the label, the sample was blended with a Vortex mixer, and [^{14}C]-methane in the headspace was analyzed by the gas chromatographic-proportion counter method described by Nelson and Zeikus (12). The sample was then acidified, and the carbon dioxide released was trapped in a toluene-based scintillation fluid containing phenethylamine as the trapping agent (17). The sediment was centrifuged, and the label in the supernatant and pellet was counted as described above for [^{35}S]methionine.

Thin-layer chromatographic analysis. Soluble labeled compounds were analyzed by one-dimensional thin-layer chromatography. Commercially prepared plastic thin-layer plates (20 by 20 cm) with a 0.1-mm layer of cellulose (Polygram CEL 300, Brinkmann Instruments Inc., Westbury, N.Y.) were used. The solvent used was 2-butanol–water–formic acid (6:2:1 [vol/vol]) (11). This solvent was made up freshly each day. The sample (1 to 5 μl) was spotted onto the plate 1.5 cm from the bottom, and the solvent front was allowed to migrate 13 cm (ca. 3.5 h) past that point. For analysis of labeled compounds, each sample was cut into 13 strips 1 cm long. Each strip was placed in a scintillation vial containing 10 ml of Aquasol scintillation cocktail and was counted as previously described. The mobilities of the radioactive compounds were compared with those of nonradioactive standards (see legend of Fig. 3), which were generally detected on the thin-layer chromatography plates by means of a ninhydrin spray (Sigma Chemical Co., St. Louis, Mo.). This solvent system, and many other ones tried, failed to separate methionine sulfoxide and methionine sulfone significantly. The inability to separate these two compounds by thin-layer chromatography has also been reported by Tonzetich (19).

Chemicals used. Methane thiol was purchased from Matheson Gas Products, East Rutherford, N.J. Dimethyl sulfide and dimethyl disulfide were purchased from Fisher Scientific Co., Fair Lawn, N.J. Carbon disulfide was purchased from Matheson, Coleman and Bell, Norwood, Ohio. All standards for thin-layer chromatography analysis (see Fig. 3) were obtained from Sigma Chemical Co. All other chemicals were of reagent grade.

Purity of radiochemicals used. When the [^{35}S]methionine preparation used (Amersham/Searle) was analyzed by the thin-layer chromatography method previously described, one-third of the radioactivity was found to comigrate with methionine sulfoxide and methionine sulfone, and the rest of the label was in the methionine fraction. These impurities are common in labeled methionine preparations (19). Often, labeled methionine preparations contain mercap-

toethanol to prevent this oxidation, but such preparations were not used because of the possible interference of mercaptoethanol with sediment sulfur metabolism. The [*methyl*-^{14}C]methionine preparation used (Amersham/Searle) contained 10 to 15% methionine sulfoxide and sulfone.

RESULTS

Degradation of unlabeled methionine. When sediments received no additions and were incubated anaerobically for 4 days at 20°C, no volatile sulfur compounds were detected in the headspace (Table 1). Furthermore, all schemes for concentrating volatile sulfur compounds, such as cryogenic trapping, failed to show the presence of any volatile organic sulfur compounds in either the sediments or the water overlying the sediments. Hydrogen sulfide was detected in the water overlying the sediments during the later stages of summer stratification and was detected in the sediments only after acidification, which dissolves ferrous suflide. Only in aerobic surface waters, where cyanobacteria were decomposing, were trace amounts (1 to 10 ng/liter) of dimethyl sulfide detected.

Chloroform added to the sediments caused the production of some methane thiol (and its oxidized form, dimethyl disulfide) and a small amount of dimethyl sulfide (Table 1). Other peaks were also found in the gas chromatographic trace from these sediments. One was positively identified as carbon disulfide, while another was tentatively identified as *n*-propane thiol, although ethyl methyl sulfide has the same retention time (4). There was also a small peak that came out roughly 60 min after injection, which may have been dipropyl disulfide (the disulfide form of propane thiol), extrapolating from the data of Banwart and Bremner (1).

When methionine was added to a final concentration of 1 μmol/ml, approximately 0.1% of the sulfur added could be accounted for as methane thiol and dimethyl sulfide. There was a stimulation of methane thiol production from methionine when chloroform was added and a decrease in dimethyl sulfide production. There was also a small stimulation of carbon disulfide production by methionine in $CHCl_3$-treated sediments; there was no effect on propane thiol production.

Because cysteine has been shown to be a source of carbon disulfide (2) and is a possible source of propane thiol, cysteine additions were made to the sediments. There was no production of volatile organic sulfur compounds from cysteine alone, and when chloroform was present, the results were similar to the control. Hydrogen sulfide, a likely product of cysteine decomposition, was not found in the headspace, probably because the ferrous iron present in the sediments precipitated any sulfide formed as insoluble iron sulfides.

Time course for degradation of [^{35}S]methionine. [^{35}S]methionine was rapidly metabolized by Lake Mendota sediments (Fig. 1). At zero time, all the label was found in the supernatant, except for a small amount (ca. 5%) that appeared to adsorb abiologically to the sediments. After 2 h of incubation, a significant fraction of the label was converted into hydrogen sulfide. A roughly equal amount of label was also incorporated into the insoluble fraction. No significant portion of the label could be freed from the sediments as volatile organic sulfur. When about one-third of the label remained in the soluble fraction, the reaction rate decreased considerably. That one-third of the [^{35}S]methionine preparation used was methionine sulfoxide or sulfone (see above) partly explains this. This phenomenon will be discussed in greater detail in a section below on thin-layer chromatography analysis of the soluble fraction.

In the experiment presented in Fig. 1, the rate of reaction was relatively high. With other sediment samples, it took as long as 8 h to reach the degree of completion reached by this sediment sample in 2 h. The usual reaction time was 4 h. Despite this difference in reaction rates, the ratio of the products to each other was always similar, as was the response of the sediments to additions. We have not been able to correlate

TABLE 1. *Volatile organic sulfur compound production by lake sediments*[a]

Sample	Sulfur (nmol)			
	CH_3SH + $(CH_3S)_2$	$(CH_3)_2S$	CS_2	C_3H_7SH[b]
Control	0.0	0.0	0.0	0.0
Control + $CHCl_3$ (750 μg/ml)	1.0	0.05	2.2	2.1
L-Methionine (1 μmol/ml)	1.5	0.7	0.0	0.0
L-Methionine + $CHCl_3$	11.2	0.3	4.0	1.8
L-Cysteine (1 μmol/ml)	0.0	0.0	0.0	0.0
L-Cysteine + $CHCl_3$	0.8	0.05	1.4	1.8

[a] Sediment (2 ml) was incubated for 96 h at 20°C under a nitrogen atmosphere.
[b] Tentative identification.

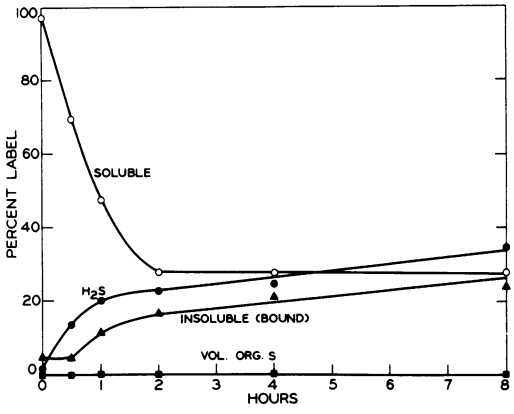

FIG. 1. *Time course for metabolism of 1.75-nmol/ml* L-*[^{35}S]methionine (1.2 × 10^6 dpm) by Lake Mendota sediments. Incubations were at 15°C under a nitrogen atmosphere.*

these reaction rate differences with seasonal changes or any other factor, and they may reflect variations in sediment characteristics at the sampling site.

Temperature optimum for [^{35}S]methionine metabolism. The demonstration of a temperature optimum is strong evidence that a reaction is biological. Both hydrogen sulfide production and incorporation of [^{35}S]methionine into insoluble material show a temperature optimum (Fig. 2). The temperature optimum in both cases (30 to 40°C) is significantly higher than the highest temperature ever reached by the sediments in situ (15°C). This is similar to the findings of Zeikus and Winfrey (22), who found a temperature optimum of 35 to 42°C for methanogenesis by Lake Mendota sediments. The small amount of incorporation of label into the insoluble fraction at both 0 and 70°C is further evidence that some abiological adsorption of methionine by sediments is taking place. No label was found as free volatile organic sulfur compounds at any temperature.

Effects of additions on [^{35}S]methionine metabolism by sediments. Various additions were made to the lake sediments to determine what effect they would have on metabolism of [^{35}S]methionine. The addition of 10-μmol/ml glucose did not affect the labeling pattern from [^{35}S]methionine (Table 2). Neither did incubation of the sediments under an atmosphere of pure hydrogen, which greatly stimulates methane production (22). However, when methane thiol was added to the headspace, there was a dramatic increase of label in the volatile organic sulfur fraction, with a corresponding decrease of label in the hydrogen sulfide and insoluble fractions. This result was repeated several times.

When nonlabeled L-methionine was added to the samples so that the final methionine concentration was 10 times (17.5 nmol/ml) or 100 times (175 nmol/ml) the original concentration, the amount of label converted to hydrogen sulfide decreased because of the lower specific activity of the label. If these rates are multiplied by the dilution factors, the actual rates increase. If the label conversion rates are plotted as a Lineweaver-Burk plot, they fall on a straight line, with an apparent K_m of 10^{-5} M. This is further evidence that the process is biological.

The addition of 175-nmol/ml L-cysteine de-

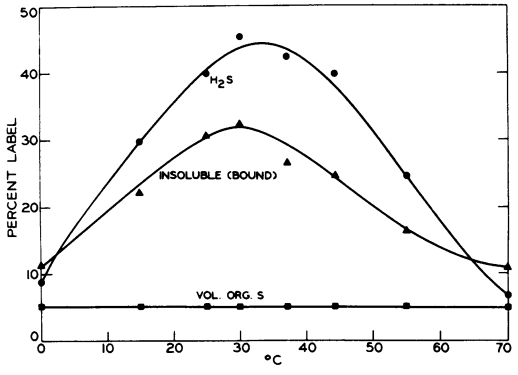

FIG. 2. *Temperature optimum for metabolism of 1.75-nmol ml* L-*[^{35}S]methionine (1.2 × 10^6 dpm) by Lake Mendota sediments. Incubations were for 2 h under a nitrogen atmosphere.*

TABLE 2. *Effects of additions on metabolism of 1.75-nmol/ml [^{35}S]methionine by lake sediments*[a]

Sample	Label (%)			
	Hydrogen sulfide	Volatile organic sulfur compounds	Insoluble	Soluble
Expt 1				
Control	33	0.2	28	34
H_2 (1 atm)	32	0.1	27	36
Glucose (10 μmol/ml)	34	0.2	34	30
CH_3SH (0.01 atm)	10	29	14	56
Expt 2				
Control	17	0.2	25	47
17.5-nmol/ml L-methionine	7.5	0.2	14	73
175-nmol/ml L-methionine	1.9	0.1	7	93
Expt 3				
Control	20	0.3	18	61
175-nmol/ml L-cysteine	9	0.3	7	94
175-nmol/ml DL-ethionine	3	0.2	7	81

[a] Samples were incubated 2 to 4 h at 15°C under a nitrogen atmosphere unless otherwise noted. Activity of [^{35}S]methionine, 1.0 × 10^6 to 1.5 × 10^6 dpm.

creased hydrogen sulfide production from methionine by half, whereas 175-nmol/ml DL-ethionine had a much more marked effect. Ethionine is a well-known inhibitor of methionine metabolism.

Metabolism of [*methyl*-^{14}C]methionine and comparison with [^{35}S]methionine. Sediments were incubated with [^{14}C]methionine labeled in the terminal methyl group. Parallel incubations were done with [^{35}S]methionine for comparison. Methane and carbon dioxide were formed from the terminal methyl group of methionine (Table 3). The sum of the percentages of label represented by methane and carbon dioxide (19%) was roughly equal to the percentage of [^{35}S]methionine label converted to hydrogen sulfide (23%). The amount of label incorporated into the insoluble fraction was also similar for both labels.

Chloroform completely inhibited labeled methane production from the terminal methyl group of methionine, although there was still some carbon dioxide production. Hydrogen sulfide production was decreased by half by the chloroform treatment, and there was no production of free volatile organic sulfur compounds in this short-term experiment. Sulfate had little effect on metabolism of either [^{35}S]methionine or [*methyl*-^{14}C]methionine, although there seemed to be some decrease in the label incorporated into insoluble material. All activity was abolished when the sediments were heated to 70°C under a nitrogen atmosphere for 1 h before incubation with label. This heat treatment did not affect the appearance of the sediments and served as a killed control.

Long-term incubation of sediments with [^{35}S]methionine. Because the incubations with nonlabeled methionine were for a much longer time period (96 h) than those using label (2 to 4 h), the results are not directly comparable. For this reason, sediments were incubated with [^{35}S]methionine for 48 h.

The labeling pattern was similar to the short-term incubations with [^{35}S]methionine (Table 4). A somewhat greater portion of the label was converted to hydrogen sulfide. Incubation of the sediments under an atmosphere of pure hydrogen did not significantly change the labeling pattern. Chloroform decreased the amount of label converted to hydrogen sulfide and slightly increased the label in the insoluble fraction. A significant portion of the label was found as free volatile organic sulfur compounds, as in the experiments that used nonlabeled methionine. Increasing the total methionine concentration 100-fold to 175 nmol/ml significantly decreased both the portion of the label converted to hydrogen sulfide and the label incorporated into the insoluble fraction, as in the short-term label experiments. However, there was production of labeled free volatile organic sulfur compounds. Methane thiol, dimethyl disulfide, and dimethyl sulfide were detected in the headspace above the sediments in concentrations comparable to those described above for long-term incubations of high concentrations of methionine (1 μmol/ml).

Thin-layer chromatography analysis of soluble label after [^{35}S]methionine metabolism by sediments. When sediments metabolize [^{35}S]methionine, the disappearance of the

TABLE 4. *Long-term (48-h) incubation of lake sediments with 1.75-nmol/ml [^{35}S]methionine in the presence of various additions*[a]

Sample	Label (%)			
	Hydrogen sulfide	Volatile organic sulfur compounds	Insoluble	Soluble
Control	38	0	28	19
H_2 (1 atm)	32	0	35	17
$CHCl_3$ (750 μg/ml)	15	6	20	38
175-nmol/ml methionine	7	6[b]	10	74

[a] Sediment was incubated at 15°C under a nitrogen atmosphere unless otherwise noted. Activity of [^{35}S]methionine, 1.5 × 10^6 dpm.

[b] Gas chromatograph detected 1.5 nmol of CH_3SH + $(CH_3S)_2$ sulfur and 0.16 nmol of $(CH_3)_2$ sulfur.

TABLE 3. *Products formed from metabolism of 1.75-nmol/ml [methyl-^{14}C]methionine and 1.75-nmol/ml [^{35}S]methionine by sediments*[a]

Sample	^{14}C (%)			^{35}S (%)		
	Methane	Carbon dioxide	Insoluble	Hydrogen sulfide	Volatile organic sulfur compounds	Insoluble
Control	10	9	24	23	0.5	29
$CHCl_3$ (750 μg/ml)	0	5	14	11	0.5	26
SO_4 (10 μmol/ml)	11	7	16	21	0.3	22
Heat killed (70°C, 1 h)	0	0.2	1	3	0.2	4

[a] Sediment was incubated 4 h at 15°C under a nitrogen atmosphere. Activities: [*methyl*-^{14}C]methionine, 2 × 10^5 dpm; [^{35}S]methionine, 1.2 × 10^6 dpm.

label from the soluble fraction was rapid until approximately one third of the label remained, after which point the amount of label in the soluble fraction did not change (Fig. 1). To better understand this phenomenon, the soluble fraction before and after metabolism was analyzed by thin-layer chromatography.

As described above, approximately one third of the [^{35}S]methionine preparation used was either methionine sulfoxide or methionine sulfone, so that the fraction of methionine in the labeled preparation was roughly equal to the fraction degraded and incorporated into insoluble material. Although methionine sulfoxide should be readily reduced to methionine by the microflora in the sediments, sulfones are very chemically stable and are reduced slowly, if at all by microorganisms (5, 19; S. H. Zinder and T. D. Brock, J. Gen. Microbiol., in press).

Another possible explanation of incomplete methionine degradation is that methionine strongly adsorbs to clay particles in the sediment and is therefore unavailable for metabolism. When acid is added, it neutralizes the charge on the clay particles, thereby releasing the methionine. Acid treatment could also lyse cells that have taken up methionine, releasing it into the supernatant. It was found that if, instead of acid, the sediments were treated with a dilute zinc acetate solution (to stabilize sulfide), there was 30% less label in the supernatant. A third explanation is that methionine is metabolized to a soluble product that is not metabolized further.

Figure 3 presents a thin-layer chromatography analysis of the soluble fraction after metabolism of [^{35}S]methionine. When the sediments were acidified, there were four peaks. Two of them were small and had mobilities corresponding with methionine (fraction 10) and homocysteine (fraction 8). There was a large peak with a mobility corresponding with methionine sulfoxide and methionine sulfone (fraction 5), and a large unidentified peak in fraction 2. When the sediments were not acidified, the peaks in fractions 8 and 10 were not present.

Thus, there appear to be multiple causes for the label remaining in the supernatant. The presence of oxidized forms of methionine in the label preparation used, the adsorption of methionine by the sediments, and the metabolism of methionine to a soluble product (the unidentified peak) all appear to contribute.

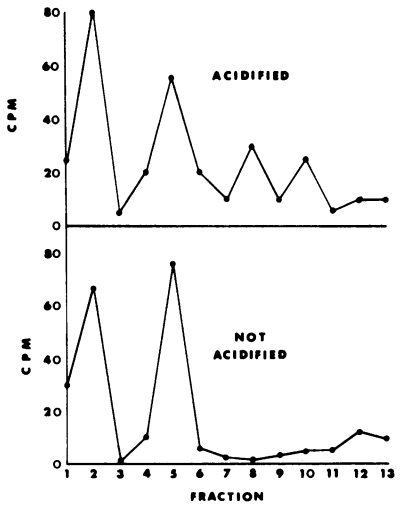

FIG. 3. *Thin-layer chromatographic analysis of the soluble label after metabolism of 1.75-nmol/ml [^{35}S]methionine by Lake Mendota sediments. The acidified sample received 1-ml/ml sediment of 3.0 N HCl, and the hydrogen sulfide was bubbled off. The non-acidified sample received 1-ml/ml sediment of a dilute zinc acetate solution to stabilize sulfide. The compounds listed would be found in the following fractions: methionine, 10; methionine sulfoxide, 5; methionine sulfone, 5; methionine sulfonium chloride, 4; homocysteine, 8 to 9; homocystine, 5; cysteine, 7; cystine, 3; α-ketoγ-methiolbutyric acid, 12.*

DISCUSSION

Lake Mendota sediments produced methane thiol, dimethyl disulfide, and dimethyl sulfide from unlabeled methionine, as has been described previously in other anaerobic habitats (8, 15). An interesting phenomenon is the production of methanethiol by sediments treated with chloroform, even when methionine was not added. We have obtained similar results with anaerobic sewage digestor sludge (Zinder and Brock, unpublished data), except that methane thiol production was much greater, as would be expected in a system that is more actively degrading protein. Possible mechanisms for this methane thiol production will be mentioned later in the discussion.

Also of interest is the production by chloroform-treated sediments of carbon disulfide and of what is tentatively identified as *n*-propane thiol. Carbon disulfide production from manure has been reported (3), and trace amounts have been detected in ocean sediments (10). Although Banwart and Bremner (2) have found cysteine to be a source of carbon disulfide in soils, we were unable to detect production of any volatile organic sulfur compound from cysteine, with or without chloroform present. The source of *n*-propane thiol is also unknown. These compounds were also produced by digestor sludge treated with chloroform, but in much lower amounts than methane thiol.

When tracer quantities of ^{35}S-labeled methionine were added to sediments, the label went

into hydrogen sulfide and insoluble material in roughly equal amounts. The incorporation of label into the insoluble fraction may have been the result of microbial uptake of [^{35}S]methionine. Another possibility is that the insoluble counts were methane thiol that had adsorbed to the sediments. Preliminary experiments with ^{14}C-labeled and nonlabeled methane thiol have shown that it is rapidly bound to sediments and is not freed by acid. Similar binding of volatile organic sulfur compounds to soils has been described (5). Attempts to determine the nature of the insoluble radioactivity by extraction have not been successful.

The products formed from the terminal methiol group of methionine were methane, carbon dioxide, and hydrogen sulfide. There are two general mechanisms by which these products can be formed. One is that described by Postgate (14), which involves cleavage of the terminal methyl group from the sulfur via *S*-adenosyl methionine; it can be represented by these equations: (i) methionine $\rightarrow$ homocysteine + $CH_3 \cdot$ carrier; (ii) $CH_3 \cdot$ carrier $\rightarrow CH_4 + CO_2$; (iii) homocysteine $\rightarrow$ homoserine + H_2S. The methyl group carrier could be tetrahydrofolate or vitamin B_{12} or other carriers. The other general mechanism involves cleavage of the whole methiol group with the production of methane thiol: (iv) methionine $\rightarrow$ homoserine + CH_3SH; (v) $CH_3SH \rightarrow CH_4 + CO_2 + H_2S$. The end products are the same in both cases.

Most of the evidence collected in this study favors the second mechanism, but it does not completely eliminate the first. The most convincing evidence for the second mechanism is the production of labeled volatile organic sulfur compounds from [^{35}S]methionine in the presence of excess methane thiol. A likely explanation for this is that, although labeled methane thiol produced from methionine usually binds to the sediments, the large amount of unlabeled methane thiol present had already saturated the binding sites. Furthermore, the labeled methane thiol produced pools with the large excess of the unlabeled compound, so that only a small fraction of the label was metabolized further to hydrogen sulfide.

Methane thiol was produced from sediments incubated in the presence of chloroform. Chloroform did not inhibit and, in fact, actually stimulated methane thiol production from unlabeled methionine (Table 1) and caused production of free volatile organic sulfur compounds from [^{35}S]methionine when sediments were incubated for 48 h (Table 4). Presumably, the longer incubation time was needed so that sediment binding sites that absorb methane thiol could be saturated and methane thiol produced could then be released. If chloroform does not block methane thiol production from methionine (equation iv), but does block sediment metabolism of methane thiol (equation v), then methane thiol would build up. Chloroform may also block metabolism of carbon disulfide and propane thiol. An alternative explanation for the action of chloroform is that it blocks the reaction in equation ii, causing a shift in methionine metabolism such that methane thiol is produced. It would be of interest to examine the effects of inhibitory analogs of coenzyme M on methionine metabolism.

The second mechanism for methionine metabolism requires that methane thiol be metabolized to methane, carbon dioxide, and hydrogen sulfide (equation v). In studies using ^{14}C-labeled methane thiol (S. H. Zinder and T. D. Brock, unpublished data), we have found that methane thiol was readily metabolized by the lake sediments to methane, carbon dioxide, and (presumably) hydrogen sulfide. Methane production from methane thiol was completely inhibited by chloroform, but there was still some carbon dioxide production, similar to the results obtained with [*methyl*-^{14}C]methionine. The metabolism of methane thiol by sediments makes the second reaction possible, but does not completely eliminate the first.

Short-term incubations with low concentrations of radiotracers are more likely to resemble the natural metabolism of methionine in the sediments. The free methionine in the sediments is most likely at a steady-state concentration even lower than the concentration we added. However, this sort of addition may be similar to the situation in which an algal cell falls near a microbial population, so that there is a localized higher concentration of methionine. Whether methionine is metabolized when it is part of a peptide is unknown. Glucose did not affect the metabolism of the small amount of [^{35}S]methionine added to sediments (Table 2), in contrast to the results of Francis et al. (8), who found that, in anaerobically incubated soils, glucose was needed for product formation from methionine.

The Lake Mendota sediments may be considered a "mature" anaerobic degradation system in which methane, carbon dioxide, and hydrogen sulfide are produced as the end products of carbon and sulfur metabolism and in which the concentrations of intermediate products are low. Usually, methane thiol is produced in habitats in which a large amount of protein degradation occurs before other populations, such as methanogens, can establish themselves to balance the metabolism. Such habitats are manures (3) and spoiling foods (9). Usually, there is buildup of

other intermediate compounds, such as fatty acids and amines. If the sediments are "overloaded" with methionine, methane thiol is produced, but if small amounts are added, only hydrogen sulfide is produced. We have never been able to detect any volatile sulfur compounds other than hydrogen sulfide associated with the sediments. Since methionine sulfur is readily metabolized to hydrogen sulfide, it is likely to be a major source of sulfide in the sediments.

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